Life Sciences, Vol. 62, No. 3, pp. PL 43–48, 1998
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0024-3205/98 $19.00 + .00

PII S0024-3205(97)01106-5

PHARMACOLOGY LETTERS

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POLYENZYME PREPARATION WOBE-MUGOS® INHIBITS GROWTH OF SOLID TUMORS AND DEVELOPMENT OF EXPERIMENTAL METASTASES IN MICE

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(Submitted July 23, 1997; accepted August 20, 1997;
received in final form October 30, 1997)

Abstract. Long-term rectal administration of enzyme mixture containing papain, trypsin and chymotrypsin in the same ratio as the preparation Wobe-Mugos E (Mucos Pharma, Germany) was evaluated for their antitumor effects in $C_{57}Bl_6$ inbred mice inoculated with $B1_6$ melanoma cells. 30% of animals in the test group (3 pcs) have been cured of cancer. In the rest of animals (70%) the survival time was prolonged by 58.3% compared to the control group (from average survival time of 24 days in control group to 38 days in the test group). Based on histological and immunohistochemical evaluation a faster process of metastasizing was found in control group than in the group treated with the polyenzyme preparation. In the case of melanoma Bl_6 an antimetastatic effect of the preparation was thus proved. © 1997 Elsevier Science Inc.

Key Words: melanoma, metastases, polyenzyme preparations, mice

Introduction

Recurrent or metastatic human malignant melanoma is difficult to cure. Conventional chemotherapeutic agents are ineffective in inhibiting the growth of melanomas in more advanced stages. It is therefore important to improve treatment modalities for human melanomas particularly in view of the increasing incidence of melanoma in recent years. Combinations of proteolytic enzymes have been currently used in clinical practice mainly because of their adjuvant effects in the course of radiotherapy and chemotherapy. They help to minimize therapy side-effects and serious complications and improve the course of final stages (8). The metastases prophylaxis by enzyme preparations has been relatively little experimentally studied and clinical investigations are not supported by sufficient experimental background. The aim of presented investigation was to study the effect of Wobe-Mugos E containing proteolytic enzymes on $C_{57}Bl_6$ inbred mice with transplanted melanoma $B1_6$.

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Methods

Animals: inbred mice $C_{57}Bl_6$, female, body weight 18-20 g (AnLab s.r.o. - Charles River, CR) were used. Mice were grown in SPF menagerie, kept on bedding sterilized by radiation (SAWI Research Bedding), fed by ST-1 chow sterilized by radiation (Bergmann s.r.o.) and autoclaved water ad libitum. Tumor cells: 2 x 10^6 of melanoma Bl_6 tumor cells were intraperitoneally transplanted into the abdominal cavity of inbred mice $C_{57}Bl_6$ where they proliferated in the form of ascites. On the day 10 after the transplantation the ascites was removed and transferred into Hanks solution, the cells were counted and diluted to the concentration of 2 x 10^6 / 0.2 ml of suspension. 20 mice ($C_{57}Bl_6$) were intradermally inoculated on the left side with this tumor cell suspension. The growing tumors were surgically removed on the day 10 after application. Day 10 was chosen based on previous experiments when a metastatic activity of melanoma Bl_6 was studied (all mice included in these experiments died because of metastases within 3 weeks after the surgical removal of primary tumor on the day 10). Treatment with proteolytic enzymes: enzyme mixture containing proteolytic enzymes in the same ratio as the preparation Wobe-Mugos E [Mucos Pharma, Germany - one tablet contains papain 100 mg (=270 F.I.P.E), trypsin 40 mg (=29 μkat), chymotrypsin 40 mg (=200 μkat), corresponding to the total proteolytic activity from 1740 F.I.P.E.] was used. Immediately after removal of primary tumor the enzyme mixture was rectally administered to the mice in test group (10 pcs) - 2 times a day, at 8 a.m. and 5 p.m., each dose containing 45 mg/kg of body weight in 0.1 ml of saline. The dosage was derived from the daily therapeutic dose (4 x 5 tablets of Wobe-Mugos E) used in human patients. The equal volume of saline was rectally administered to the control group (10 pcs). Treatment of both, test and control, groups of mice was carried out continuously till the decease of animals. Eventual decease was followed daily (including Saturdays and Sundays) for 100 days. A complete obduction of each deceased animal was done. Organs afflicted by metastases were transferred into Bouin's fixation solution for immunohistochemical and histological evaluation. Histology samples were prepared using standard techniques and studied under optical microscope. Samples for immunohistochemical study: slices were dehydrated by xylene and decreasing line of alcohols, predigested by trypsin and washed with PBS. Endogenous peroxidases were then blocked by hydrogen peroxide, washed and an antibody S 100 (S 2644, Sigma Immunochemicals) - monoclonal antibody conjugated with peroxidase - was applied. After the washing a chromogen with substrate - DAB (diaminobenzidine) and hydrogen peroxide were applied. After the reaction samples were again stained by hematoxyline-eosine, immersed into the Canadian balsam and evaluated under optical microscope (5, 7).

Results

Obtained results are depicted in Figure 1. 30% of animals (3 pcs) from test group survived for 100 days. They were killed on the day 101. Histological and immunohistochemical examination showed no cancer processes. The rest of animals (70%, 7 pcs) survived on the average by 58.3% longer than mice in control group (10 pcs). An average survival time was prolonged from 24 days in control group to 38 days in the rest of animals from test group.

Obduction of individual deceased animals in test group showed:
1. Enlarged axillary nodes, in some cases with macroscopically visible metastases (on the left side).
2. Chest cavity filled with metastases, eventually only visible metastases in lungs.
3. Enlarged spleen.

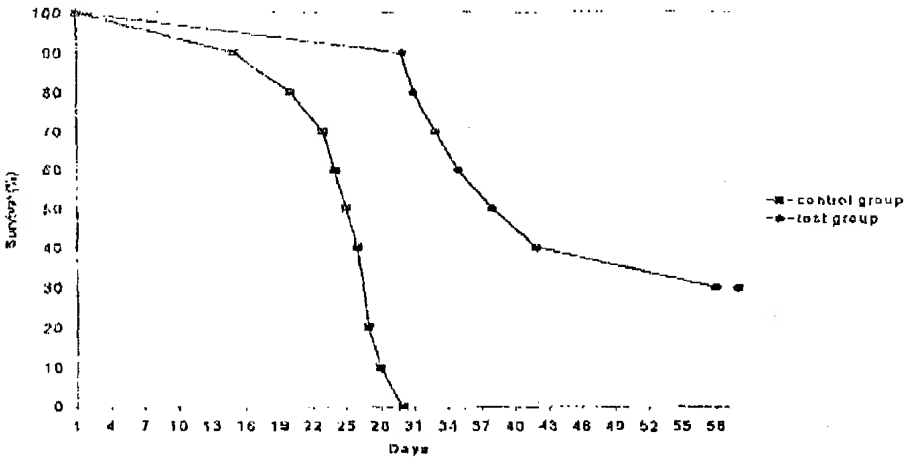

Fig. 1
Survival of mice $C_{57}Bl_6$ after therapy with Wobe-Mugos E

Obduction findings were the same in both, control and test, groups. In one case metastases were found in mesenteric nodes of small intestine.

Histological evaluation:
In the lung parenchyma of both, control and test, groups secondary tumor foci of irregular shape and size were observed. Nodal formations (size 0.5–5 mm) compressed surrounding tissue. Tumor cells were atypical, of polymorphic shape, either hyperchromnic or vesiculiform nuclei with marked nucleolei. At some places cells of spindle shape similar to sarcoma could be seen. In the cells a increased number of mitoses and small amount of pigment were found, too. The boundary of melanoma was not precise, tumor cells disturbed a structure of original tissue. Larger tumors exhibited foci of hemorrhages and necroses (Fig. 2a, 2b). Other metastases were observed in lymphatic nodes under the scapula, sporadically in pericardium and in the kidney capsule. The results suggest a selective way of metastasis, since the lung tissue is systematically attacked. Liver, spleen, kidney, and myocardium were finding-free.

By comparison of findings in test and control groups it could be concluded that metastasizing was faster in control group. In studied samples taken at the same time more developed tumor changes (larger tumor foci accompanied by necroses) were detected in control group.

Immunohistochemical evaluation:
In both, control and test, groups melanoma cells reacted with antibody. The cell labeling in immunoperoxidase reaction was perceptible always in cells proliferating on the edges of tumor foci of lung tissue. Immunohistochemical findings supported histological evaluation in the localization of metastases (Fig. 3a, 3b). More extensive findings were always encountered in control group, whereas in the test group smaller metastatic foci were found. Occasional tiny incipient foci could be observed in lung tissue. No labeled cells were found in liver, spleen, kidney, and myocardium.

Fig. 2
(a) Test group - lungs: disseminated larger and smaller irregular tumor foci, predominantly in vessel areas. Polymorphic cells with vesiculiform nucleus and nucleolus. Sporadic occurrence of polymorphic cells. (b) Control group - lungs: regressive changes (hemorrhages and necroses) can be observed in tumors. Tumor cells proliferate on the edge.

Fig. 3
(a) Test group - lungs: proliferating cells in lung tumor foci are actively labeled by peroxidase. (b) Control group - lungs: proliferating cells on the edge of necrosing tumor foci in lungs are labeled by peroxidase.

The results described here demonstrate that the polyenzyme preparation Wobe-Mugos E is capable to inhibit Bl_6 melanoma tumor growth and metastasizing in mice.

Discussion

Proteolytic enzymes (e.g. bromelain, papain, trypsin) have been used for a long time in prevention of metastases and also as a part of adjuvant therapy in cancer treatment (17, 19). Enzyme preparations reduced side effects of bleomycin treatment in patients with head and neck cancer (13), improved the tolerance of radiation in abdominal cancer patients (14) and reduced severity of mucositis after radiation (16). The remission time of patients with multiple myeloma treated additionally to chemotherapy with Wobe-Mugos E was significantly longer (10, 11, 12).

Direct application of Wobe-Mugos E into melanomas reduces tumor growth and metastatic formation (Scheef personal communication). Wobenzym, Wobe-Mugos E and its constituents

reduce the expression of the vitronectin receptors in human melanoma cell lines in vitro (4). The attachment of murine Bl_6 melanoma cells to the complete matrix (ECM) is inhibited by 40% after treatment with bromelain. This enzyme reduces the RGD attachment (15). The CD44 expression on lymphocytes is also modulated by enzymes (7). Integrins but also CD44 are involved in metastatic formation, not only in melanomas. Since the enzyme preparations reveal the capacity to modulate the expression of adhesion molecules a possibility for their therapeutic use in adjuvant therapy of melanomas can be suggested.

Direct molecular mechanism of enzyme effect on prophylaxis of metastases has not been fully clarified yet . A decrease of immune complexes (18), disruption of adhesion molecules on tumor and endothelial cells (4, 7), degradation of cytokines and cytokine receptors (11, 12) and also positive immunomodulating effect (3, 20) were described. Studies on laboratory animals have shown an antimetastatic effect of bromelain in rats with Yoshida sarcoma (1). Additional evidence exists that proteolytic enzymes such as bromelain (Ananaes comosus extract) may play a role in differentiation processes of malignant cells and diminished the tendency to form a pulmonary metastasis in mice (Lewis lung carcinoma) (2).

In contrast to Batkin et al. (1988) in presented experiment the exact dose of the preparation was administered to each animal via probe - rectal. Occurrence of metastases was evaluated by a complex histological and immunohistochemical examination. In agreement with previous studies (9) an obvious antimetastatic effect of enzyme preparation Wobe-Mugos E was found. Primary aim of described study was to focus on verification of assumed antimetastatic effect of Wobe-Mugos E on melanoma Bl_6 growing on $C_{57}Bl_6$ inbred mice. The authors tried to simulate situation as close to clinical experience as possible. Therefore, a growing tumor was surgically removed on the day 10 after the transplantation and a development of metastases was observed.

From the results of presented study it can be concluded that the polyenzyme preparation Wobe-Mugos E has the capacity to prevent and inhibit Bl_6 melanoma tumor and metastatic growth in mice.

These studies are an important first step towards further experiments with polyenzyme preparation and melanoma development , which, we believe, will provide new chance for cancer therapy. To confirm this positive result repetition of the experiment using a large group of animals is planed.

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